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The toxicological significance of post-mortem drug concentrations in bile

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Keywords: Bile; drug bile concentration; drug blood concentration; ethanol; opioids; post-mortem toxicology

Abstract

Context: Some authors have proposed that post-mortem drug concentrations in bile are useful in estimating concentrations in blood. Both the International Association of Forensic Toxicologists (TIAFT) and the US Federal Aviation Administration recommend that samples of bile should be obtained in some circumstances. Furthermore, standard toxicological texts compare blood and bile concentrations, implying that concentrations in bile are of forensic value.

Aim: To review the evidence on simultaneous measurements of blood and bile drug concentrations reported in the medical literature.

Methods: We made a systematic search of EMBASE 1980–2016 using the search terms (“bile/” OR “exp drug bile level/concentration/”) AND “drug blood level/concentration/”, PubMed 1975–2017 for (“bile[tw]” OR “biliary[tw]”) AND (“concentration[tw]” OR “concentrations[tw]” OR “level[tw]” OR “levels[tw]”) AND “post-mortem[tw]”, and also MEDLINE 1990–2016 for information on drugs whose biliary concentrations were mentioned in standard textbooks. The search was limited to human studies without language restrictions. We also examined recent reviews, indexes of relevant journals, and citations in Web of Science and Google Scholar. We calculated the bile: blood concentration ratio. The searches together yielded 1031 titles with abstracts. We scanned titles and abstracts for relevance, and retrieved 230, of which 161 were considered further. We excluded 49 papers because: the paper reported only one case (30 references); the data referred only to a metabolite (1); the work was published before 1980 (3); the information concerned only samples taken during life (10); or the paper referred to a toxin or unusual recreational drug (5). The remaining 112 papers provided data for analysis, with at least two observations for each of 58 drugs.

Bile: blood concentration ratios: Median bile: blood concentration ratios varied from 0.18 (range 0.058–0.32) for dextromoramide to 520 (range 0.62–43 000) for buprenorphine. Median bile concentrations exceeded blood concentrations by one order of magnitude for several drugs, including dihydrocodeine, quetiapine, and

sildenafil; and by two orders of magnitude of for buprenorphine, colchicine, and 3,4-methylenedioxy-methamphetamine (MDMA), among others. The minimum and maximum values for the ratio differed by a factor of three or more in three-quarters of the cases where data were available, and by a factor of 10 or more for over half of the analytes.

Limitations: The data were difficult to find. Medline does not explicitly index the term “drug bile concentration”. It may well be that other reports exist, although they would not alter our major conclusion. Many of the papers that contributed data failed to specify the source of the blood samples or the post-mortem interval, so that no judgment was possible regarding post-mortem redistribution in whole blood or bile.

Conclusions: For most drugs there are wide ranges of bile:blood concentration ratios, which means that bile and blood concentrations are generally poorly correlated. Bile concentration measurements cannot readily be used to establish post-mortem blood concentrations; nor can they be extrapolated to ante-mortem concentrations. However, because drug concentrations in bile often exceed those in blood, bile may allow qualitative identification of drugs present, even when the blood concentration is below the limit of detection.

Context

The quantitative determination of drug concentrations after death can be of great forensic importance, and may lead towards or away from a diagnosis of fatal poisoning [1,2]. Most often, drug concentrations are determined in blood, ideally obtained from the femoral or popliteal vein, although the nature of the matrix changes considerably after death, and there is no assurance that post-mortem concentrations reflect those that were present before death [3–7]. Indeed, differences among blood sampling sites and changes with time after death have been labelled ‘a toxicological nightmare’ [8]. Other body tissues, notably vitreous humour, may be less susceptible to post-mortem changes [9].

Two leading textbooks of analytical toxicology list biliary concentrations of several drugs, implying that bile may be valuable for quantitative post-mortem analysis [10,11]. Both the International Association of Forensic Toxicologists [12] and the US Federal Aviation Administration [13] recommend that samples of bile should be obtained in some circumstances. However, it appears intrinsically unlikely that post-mortem concentrations of drugs in the bile will bear a constant relationship to concentrations in the blood, since even during life values in bile and blood bear no constant relationship. For example, a study of the biliary excretion of the antibacterial agent ofloxacin yielded a median bile:serum concentration ratio in six subjects studied at four times after administration that varied over a 570-fold range (0.01–5.7; mean 2.4) [14].

Two reviews published in 2016 have examined this question [15,16]. Tominaga et al. [16] provided information on bile:blood ratios in a series of over 1000 forensic autopsies, and for 15 drugs gave ranges, average values, and regression equations. They concluded that they had ‘demonstrated significant correlations for several

drugs, suggesting that bile can provide useful supplemental data in routine forensic toxicology...'. However, there was at least a five-fold difference between maximum and minimum bile:peripheral blood ratios for all the 15 drugs described. Bévalot et al. [15] conducted a careful systematic review of the literature, with 278 references and data on 133 compounds. Many of the references were to single case reports, and there was therefore no information on the likely inter-subject differences in bile:blood concentration ratios. These authors stressed that 'There are gaps in our knowledge of bile excretion, and a lack of robust studies of the interest, limitations and implementation of bile analysis in forensic toxicology;' but they saw 'a possible quantitative interest [in analysis of biliary concentrations] in the case of several molecules' [15]. In an earlier review, Vanbinst et al. [17] noted that bile was useful for analysis because 'concentrations of drugs or their metabolites are generally several-fold higher than their blood concentrations'.

Early studies on post-mortem concentrations of ethanol in blood and bile suggested that they are similar, and led to the belief that bile concentrations might be helpful in estimating blood concentrations when they are not available. For example, Stone & Rooney [18] proposed that 'a reliable prediction [sic] for a blood alcohol concentration can be given if the bile alcohol is greater than [100 mg/dL]'.

If bile concentrations bore a constant relationship to blood concentrations measured at the same time, they could be useful when, through exsanguination, coagulation, or dehydration, blood was not available. If, however, the relationship was very variable from one subject to another, quantification in bile would be unlikely to give forensic guidance. We have therefore examined the literature to discover information on bile:blood concentration ratios in post-mortem specimens. We have

supplemented a previous survey [19] with references from the two major reviews described above.

Methods

We searched the databases EMBASE and Medline from 1980 to 30 September 2016 and PubMed to January 2017 (earliest reference dated 1975), using the search strategies shown in Table 1.

{Table 1 near here}

We expanded the search by examining the review by Bévalot et al.[15], reference lists of relevant papers, searching the indexes of Forensic Science International, the Journal of Analytical Toxicology, and the Journal of Forensic Sciences, and by citation tracking in the databases Web of Science and Google Scholar. Awareness of sampling and analytical difficulties has increased over time, and we have therefore omitted studies published before 1980. For example, a large study of post-mortem total morphine concentrations provided data on 24 paired samples of bile and blood, with ratios in the range 0.5–3500 [20]. The site of blood sampling was not specified, and the study relied on fluorescence spectrophotometry to quantify morphine.

We identified 1031 papers. We scanned titles and abstracts for relevance, and retrieved 230, of which 161 were considered further. We excluded 49 papers because: the paper reported only one case (30 references); the data referred only to a metabolite (1); the work was published before 1980 (3); the information concerned only samples taken during life (10); or the paper referred to a toxin or unusual recreational drug (5). The remaining 112 papers provided data for analysis, with at

least two observations for each of 58 drugs. When information on only one patient was available, the reference was retained only if further data on the same drug were also available. Where possible, blood concentrations measured in femoral venous or “peripheral” samples were used in the comparison.

Bile:blood concentration ratios

Bile:blood ratios are tabulated as medians and ranges when relevant data were available; when the data were quoted in the originals as means and standard deviations, we converted them to means and 95% confidence intervals (Table 2). Values are given to two significant figures, to avoid spurious precision. In many cases, especially in earlier papers, the site of blood sampling was unclear, and few authors reported time from death to sampling or from sampling to analysis. The sampling site is noted when it was stated; for some analytes there were data on central or heart blood samples (which were considered together) and peripheral or femoral blood samples (again, considered together).

{Table 2 near here}

The most striking result is the very wide range of bile:blood concentration ratios for most analytes. For more than half of the 58 tabulated drugs, the minimum and maximum values for the ratio differed by a factor of 10 or more. For 74% of the analytes, the minimum and maximum ratios differed by a factor of three or more. If the bile:blood concentration ratio can often vary by one or more orders of magnitude, little reliance can be placed on any extrapolation from biliary concentrations to blood concentrations.

There are several reasons for these discrepant findings. They are listed in Table 3 under three headings: (a) physiological and pharmacological factors, (b) pathological factors, and (c) analytical factors.

{Table 3 near here}

In addition to the problems inherent in determining a fixed bile:blood drug concentration ratio, there are difficulties in interpreting results based on old analytical methods, which may be non-specific or fail to distinguish between drug and metabolite, and where specimens have been inadequately specified, for example, as 'blood'.

The previous assertion that bile concentrations of ethanol may be helpful in estimating the blood ethanol concentration is contradicted by the example given by Stone and Rooney [18]. In particular, they cite the case of a victim of a motor cycle crash whose post-mortem ethanol concentration was 220 mg/dL (0.22% or 48 mmol/L) in bile, but only 20 mg/dL (0.02% or 4.3 mmol/L) in a simultaneous blood sample. Nevertheless, the authors back-extrapolated the blood ethanol concentration to be 200 mg/dL (44 mmol/L) at the time of the crash, arguing that, 'In this case, perhaps the bile remained in the gallbladder because no digestion was taking place...'. We strongly agree with Winek et al. [21], who stated in 1983 that 'The wide range observed makes it undesirable to use bile ethanol concentrations to predict specific blood ethanol concentrations.'

Few drugs have an average bile:blood concentration ratio less than one, and for about half the included drugs, the minimum value of the ratio exceeds one. For those

drugs—which include cannabinoids, fentanyl, and morphine—whose average bile:blood concentration ratio exceeds 10, it may be convenient to screen bile, since the higher concentration potentially makes qualitative detection easier; but even for fentanyl and morphine, ratios below 1 have been reported in some cases.

Limitations

The major limitations of our review lie first in discovering the data and secondly in interpreting such data as have been published.

For example, Medline does not explicitly index the term ‘drug bile concentration’, making its contribution less useful. It may well be that other reports exist, although they would not alter our major conclusion—namely, that observed bile:blood concentration ratios vary widely between individuals and among drugs.

There are also problems in interpreting the published data. For example, many of the papers that contributed data failed to specify the source of blood samples or the post-mortem interval, so that no judgment was possible regarding post-mortem redistribution in whole blood or bile. Femoral blood [22], particularly femoral venous blood [23], or popliteal venous blood [24] is regarded as the optimum specimen for toxicological analysis. The effects of post-mortem redistribution are more apparent in the central compartment, which may explain, for example, why the maximum ratio for fentanyl in femoral blood was 12 times greater than the minimum ratio, while in heart blood it was 20 times greater.

Conclusions

The wide ranges of bile:blood concentration ratios within and between drugs show that bile and blood concentrations are generally poorly correlated. Bile concentration

measurements cannot readily be used to establish post-mortem blood concentrations or to extrapolate to ante-mortem concentrations. However, drug concentrations in bile often exceed those in blood, so that bile may allow qualitative identification of drugs present in blood but below the limit of detection.

Disclosure statement

Both authors have provided medicolegal reports on matters related to forensic pharmacology. They have no other relevant interest to declare.

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